# Researchable Question

What concentration of silk solution would be best suited to make a scaffold modeling the natural extracellular matrix of muscle tissue?

# Hypothesis

Increasing the concentration of silk in a silk solution will create a rigid scaffold.

# Purpose

The biomaterials used for cell scaffolding greatly affects the mechanical properties of the scaffold. The mechanical properties of these scaffolds are the key factors that govern the regeneration rate of the cells inside scaffolds. As of now, there exists no good way of determining which scaffolds are best suited for certain tissue regeneration. My goal is to analyze the mechanical properties of scaffolds created from various solutions of biomaterials and determine which solution is best suited for the regeneration of certain tissues.

# Background

Tissue damage is one of the leading causes of hospitalization globally, right after infection. Tissue damage can be split into two types- autonomously reparable and irreparable injuries. For irreparable tissue damage, transplants are generally used. However, the problem with transplants is that compatible donors are very difficult to find. Another emerging technique of repairing these autonomously irreparable injuries is tissue engineering. Tissue engineering uses three major components: cells, growth factors and scaffolds. My major focus is on scaffolds, the frameworks on which cells grow on. The mechanical properties of scaffolds are key properties which govern the rate at which cells regenerate inside the scaffold. Scaffolds must have mechanical properties similar to the surrounding tissue so that the resulting regenerated tissue will also be similar to the original tissue.

# Materials

- Petri dish
- Chicken leg sample
- Scalpel
- Forceps
- SDS
- Centrifuge tubes
- 6% Stock Silk Solution
- Lyophilizer
- Autoclave
- Deionized Water
- 24-well plate

# Silk Scaffold Preparation

First, a 6% stock solution provided by Coburn Lab was diluted into 5mL of 1%,2%,3%,4%,5% and 6% solutions. The diluted solutions were taken, and 1 mL of each solution was pipetted into a well of the 24-well plate. This was repeated 4 times for each concentration. Then, the solutions were lyophilized by a trained professional in the lab. After lyophilizing the silk solutions, the resulting scaffolds were autoclaved to become insoluble. The insoluble scaffolds were stored in a vacuum chamber until they were ready to be used. The scaffolds were then rehydrated in deionized water for 15 minutes. Finally, the scaffolds were cut to a height of 3mm and biopsy bunched with a diameter of 1.5mm and stored in centrifuge tubes with water in them.

# Chicken Decellularization

In order to decellularize the chicken, a 35 mL 1% Sodium Dodecyl Sulfate (SDS) solution was made. Chicken samples, cut with a radius of about 3cm were put into the SDS solution. Some chicken samples were placed in water as a control group. The solution was then put on a shaker and shaken for 72 hours. However, after the 72 hours of shaking, the extracellular matrix did not get decellularized completely, so another solution of 1% SDS and 1% bleach was made. This solution was shaken for another 72 hours, completely decellularizing the chicken.

# Constants and Variables

The independent variable for my experiment was the concentrations of silk in the silk solutions. The dependent variables for the experiment were the mechanical properties, such as Young's modulus and the stress/strain values produced by the Universal Testing Machine. All other variables were kept constant, such as temperature and material.

# Sources of Error

One major source of error was the fact that not all samples were the same size. Both types of chicken samples, the decellularized chicken and the control chicken, were very difficult to cut. Because the sample were different sizes, the stress calculations may not have been accurate, since stress is surface area dependent. Also, when decellularizing the chicken, a bleach solution was used to speed up the process. However, this may have damaged the extracellular matrix, making the extracellular matrix softer than it naturally should be.

# Data

*Table 1: Example of data collected converted to stress and strain*

| Sample 1 | | Sample 2 | | Sample 3 | | Sample 4 | | Sample 5 | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Stress ($N/mm^2$) | Strain | Stress ($N/mm^2$) | Strain | Stress ($N/mm^2$) | Strain | Stress ($N/mm^2$) | Strain | Stress ($N/mm$ |
| 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 0.0027 | 0.0004 | 0.0031 | 0.0009 | 0.0034 | 0.0024 | 0.0030 | 0.0010 | 0.0029 | 0.0006 |
| 0.0055 | 0.0014 | 0.0061 | 0.0020 | 0.0067 | 0.0049 | 0.0060 | 0.0020 | 0.0057 | 0.0016 |
| 0.0082 | 0.0025 | 0.0092 | 0.0030 | 0.0101 | 0.0074 | 0.0090 | 0.0030 | 0.0086 | 0.0025 |
| 0.0109 | 0.0035 | 0.0123 | 0.0041 | 0.0135 | 0.0099 | 0.0120 | 0.0040 | 0.0114 | 0.0035 |
| 0.0137 | 0.0046 | 0.0153 | 0.0052 | 0.0169 | 0.0124 | 0.0149 | 0.0050 | 0.0143 | 0.0044 |
| 0.0164 | 0.0056 | 0.0184 | 0.0063 | 0.0202 | 0.0149 | 0.0179 | 0.0060 | 0.0172 | 0.0054 |
| 0.0191 | 0.0066 | 0.0215 | 0.0074 | 0.0236 | 0.0174 | 0.0209 | 0.0070 | 0.0200 | 0.0064 |
| 0.0219 | 0.0077 | 0.0246 | 0.0085 | 0.0269 | 0.0199 | 0.0239 | 0.0080 | 0.0229 | 0.0073 |
| 0.0246 | 0.0087 | 0.0276 | 0.0096 | 0.0303 | 0.0224 | 0.0269 | 0.0090 | 0.0258 | 0.0083 |
| 0.0273 | 0.0097 | 0.0307 | 0.0107 | 0.0337 | 0.0250 | 0.0299 | 0.0100 | 0.0286 | 0.0093 |
| 0.0301 | 0.0108 | 0.0338 | 0.0118 | 0.0371 | 0.0275 | 0.0329 | 0.0110 | 0.0315 | 0.0102 |
| 0.0328 | 0.0118 | 0.0368 | 0.0129 | 0.0404 | 0.0300 | 0.0359 | 0.0120 | 0.0343 | 0.0112 |
| 0.0356 | 0.0129 | 0.0399 | 0.0139 | 0.0438 | 0.0325 | 0.0388 | 0.0130 | 0.0372 | 0.0122 |
| 0.0383 | 0.0139 | 0.0430 | 0.0150 | 0.0471 | 0.0350 | 0.0418 | 0.0140 | 0.0401 | 0.0131 |
| 0.0410 | 0.0149 | 0.0461 | 0.0161 | 0.0505 | 0.0375 | 0.0448 | 0.0150 | 0.0429 | 0.0141 |
| 0.0438 | 0.0160 | 0.0491 | 0.0172 | 0.0539 | 0.0400 | 0.0478 | 0.0160 | 0.0458 | 0.0150 |
| 0.0465 | 0.0170 | 0.0522 | 0.0183 | 0.0573 | 0.0425 | 0.0508 | 0.0170 | 0.0486 | 0.0160 |
| 0.0492 | 0.0180 | 0.0553 | 0.0194 | 0.0606 | 0.0450 | 0.0538 | 0.0180 | 0.0515 | 0.0170 |

# Graphs

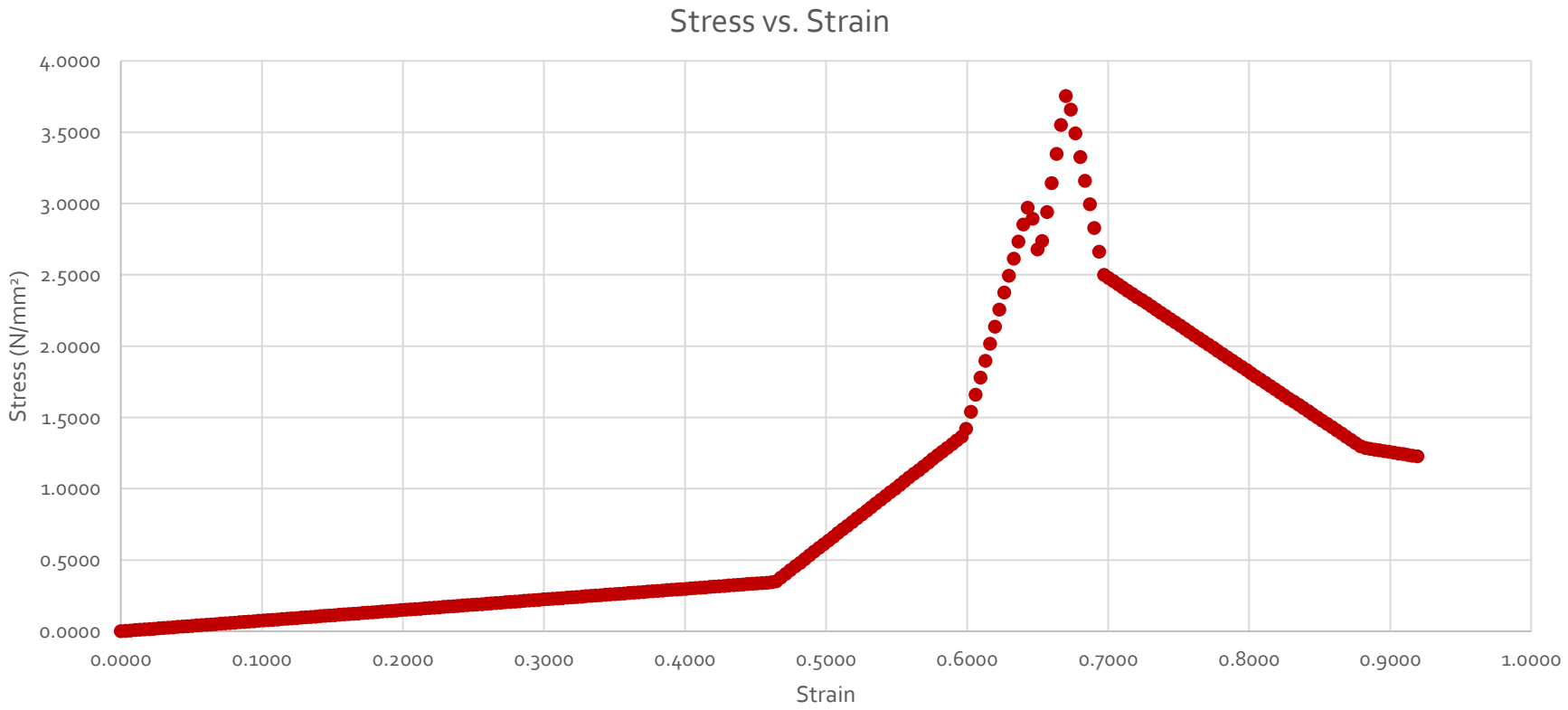


*Figure 1: A model stress vs strain graph produced by testing scaffolds*

# Data Analysis

The data will be analyzed using a distance correlation test. Then, by comparing the distance correlations between the sets of data, similarities between the sets of data can be found. Also, the moduli of the sets of data can be compared to find which samples have the most similar moduli to the decellularized data set.

# Future Work

I plan to test different concentrations of collagen, different molecular weights of silk, and different concentrations of collagen and silk samples.

# Timeline

Tuesday January 9: Finish mechanically testing collagen concentrations

Tuesday January 23: Finish mechanical testing for different silk molecular weights

Tuesday February 6: Finish testing different Collagen-Silk solutions

Thursday February 14: February Fair